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# Use of Exposure Units for Estimating Aquatic Toxicity of Organophosphate Pesticides

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Environmental water concentrations of organophosphate pesticides can be expected to fluctuate widely due to use patterns and rapid hydrolysis. This study investigated some relationships of exposure concentration, duration and periodicity to the chronic toxicity of diazinon to flagfish (Jordanella floridae). Effects were compared on the basis of "exposure units" (exposure concentration x exposure duration). Treatments at equivalent exposure units caused similar overall effects on the test populations regardless of the life stages exposed or exposure duration per se. The hypothesis is proposed that exposure units could be used to estimate the environmental impact of fluctuating water concentrations of organophosphate pesticides over a wide range of concentration, duration, and periodicity.

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| Organic phosphates* | Chronic toxicity | |
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| Toxicity* | Flagfish | 7C |
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# USE OF EXPOSURE UNITS FOR ESTIMATING AQUATIC TOXICITY OF ORGANOPHOSPHATE PESTICIDES

by

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## FOREWORD

Our nation's fresh waters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota, develops methods, conducts laboratory and field studies, and extrapolates research findings

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man.

This report describes the results of a preliminary study of the relationships of exposure concentration, duration, and periodicity to the toxicity of an organophosphate pesticide. A hypothesis is proposed for estimation of aquatic environmental impact over a range of exposure conditions.

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## ABSTRACT

This study investigated the relationship of exposure duration, concentration, and periodicity with regard to the toxicity of one organophosphate insecticide to a freshwater fish. Flagfish (*Jordanella floridae*) were exposed to uniform levels of diazinon throughout one and a half generations to establish baseline chronic toxicity values. Three additional tests were conducted with concentrations increased and duration decreased fourfold. These exposures were administered as a single pulse in each test to newly hatched, pre-spawning, and spawning flagfish, respectively. Subsequent effects on population success were observed until parents and progeny corresponded in age to the one and a half generations of the baseline test. To facilitate comparison, the toxicant challenges administered in each test were expressed as the product of exposure concentration and exposure duration ("exposure units").

All four types of exposure had similar overall effects on population growth and survival at equivalent exposure units. However, initiation of exposure during spawning caused temporary but complete inhibition of reproduction at concentrations which did not produce this effect in fish exposed continuously since hatch. This could have a severe impact on other species with a restricted reproductive period.

This study suggested the hypothesis that exposure units can be used in conjunction with previously established toxicity data to assess the environmental impact of fluctuating water concentrations of organophosphate pesticides over a wide range of concentration, duration, and periodicity.

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## SECTION I

## INTRODUCTION

During the last decade many studies have been made of the long-term effects on aquatic organisms of continuous exposure to toxicants throughout complete or partial life cycles. However, pesticide concentrations in natural waters fluctuate widely because of use patterns (Bradshaw et al., 1972) and variation in the physical, chemical, and biological characteristics of the water itself (Gomie et al., 1969; Cowart et al., 1971; Sethunathan and Pathak, 1972).

Periodicity of exposure seems to influence the toxicity of some pollutants. Acute toxicity of fluctuating levels of ammonia or zinc equaled that of constant-concentration exposure if the cycle times were short. Extending the cycle period increased the toxicity of ammonia but not of zinc (Brown et al., 1969). Allison et al. (1963) reported accretion of tissue residues and progressive chronic toxicity over a period of 20 months in trout exposed for one-half hour every 4 weeks to near-lethal concentrations of DDT. A similar study by Allison (unpublished data) with the organophosphate pesticide malathion failed to produce conclusive chronic effects. However, continuous exposure to low levels of malathion will produce chronic effects (Mount and Stephan, 1967; Eaton, 1970).

The influence of duration and periodicity of exposure on chronic toxicity became a pertinent consideration following toxicity tests with diazinon (Allison and Hermanutz, unpublished data). Continuous exposure caused chronic effects in fathead minnows and brook trout at concentrations less than $10^{-3}$ times their 96-hr LC50's. This great disparity makes the setting of a maximum permissible environmental level from the results of long-term continuous exposures to some toxicants unreasonable. A standard so derived would ignore the influence of exposure duration on the toxicity of a relatively non-persistent pesticide when the allowed maximum concentration probably would persist for only part of the life cycle of many aquatic species.

The transport, distribution, and persistence of organophosphate pesticides are subject to a large number of variable and generally unquantified factors. Estimates of exposure profiles under field conditions can be expected to have wide confidence limits. In addition, it would be impossible to simulate all probable field combinations of pesticide concentration and exposure duration in tests. Therefore, the current study was designed to compare in general the relative environmental impact of long-term, low-concentration exposure to a "non-persistent pesticide" with that of shorter term, higher concentration exposures intermediate between the currently standard acute and chronic toxicity tests. The possibility that major

differences might follow exposures limited to different life stages was included in the experimental design. It was also hoped that insight might be gained into any basic time-concentration relationship for the toxicity of organophosphate pesticides. However, no attempt was made to develop an experimental design capable of distinguishing and quantifying small differences for specific responses (growth, survival, egg hatch, etc. per se).

The purposes of this paper are: (1) To note some problems inherent in assessments of the environmental hazards of "non-persistent" pesticides; (2) to present the method used to investigate the time-concentration relationship of organophosphate-pesticide toxicity; and (3) to present the hypothesis suggested by the results of the study.

## SECTION 2

## CONCLUSIONS

Similar overall responses displayed at equivalent exposure units independent of the life stages exposed and exposure concentration or duration *per se*, lead to the conclusion that use of exposure units might permit calculation of organophosphate pesticide toxicity over a wide range of water concentration, duration, and periodicity. Such a hypothesis would have important application to estimating the impact of organophosphate pesticides in practical field situations but it requires further testing and evaluation to verify its utility.

## SECTION 3

## RECOMMENDATIONS

The hypothesis that the exposure-unit concept can be used in estimating the impact of pesticides on aquatic populations rests on a limited data base. Therefore, the results of this study should be verified for other organophosphate pesticides, other species of fishes having longer egg-to-egg life spans, and aquatic invertebrates.

If acute toxicity tests are used to formulate water-quality criteria for short-term contamination by non-persistent pesticides, the possibility of residual chronic effects on survivors should be considered in addition to concurrent mortality.

## SECTION 4

## METHODS

### EXPERIMENTAL DESIGN

Baseline chronic effects of several concentrations of the organophosphate insecticide diazinon on the flagfish (*Jordanella floridae*) were determined by continuous exposure throughout one and a half generations. The time factor involved was included by conversion to "exposure units" (exposure concentration x exposure duration) to permit comparison with the "challenge" experienced in exposures of shorter duration.

Three tests in which only a part of the life cycle was exposed used exposure units equivalent to the baseline chronic test (i.e., the same amounts of diazinon used in each level of the baseline chronic were delivered in a shorter period of time by increasing concentration accordingly). These tests were designed so that the highest concentration experienced was about two-thirds the level known to be acutely toxic. To avoid shock from sudden exposure to near-acute concentrations and to approximate a profile of environmental contamination, maximum test concentrations were built up over a period of a few days and then decreased more slowly at termination of exposure. The result was a regimen in which concentrations were increased from zero to maximum in four equal daily increments. Maximum levels were maintained for 12 days and then reduced to zero in four equal decrements at 2-day intervals. Therefore, exposures in each test consisted of single pulses of diazinon delivered over a period of 21 days (Figure 1). These pulse exposures were administered to the separate test populations at 1, 29, and 66 days post hatch, respectively (Figure 2). The pulse tests were identified by the approximate life stage exposed as larval-juvenile, juvenile-adult, and adult-spawning. Post-exposure observations were made of the impact on treated parents and their untreated progeny to an age comparable to the baseline-chronic-test fish. In the adult-spawning test the progeny reared during exposure as well as the post-exposure progeny of treated parents were observed.

Details of test techniques are given in the appendix.

### STATISTICAL ANALYSIS

The indices chosen to reflect population impact were parental survival and growth, egg production and hatch, and progeny survival and growth. The tests were run sequentially with different generations or spawnings from a common stock. Much of the extraneous variance inherent in sequential testing

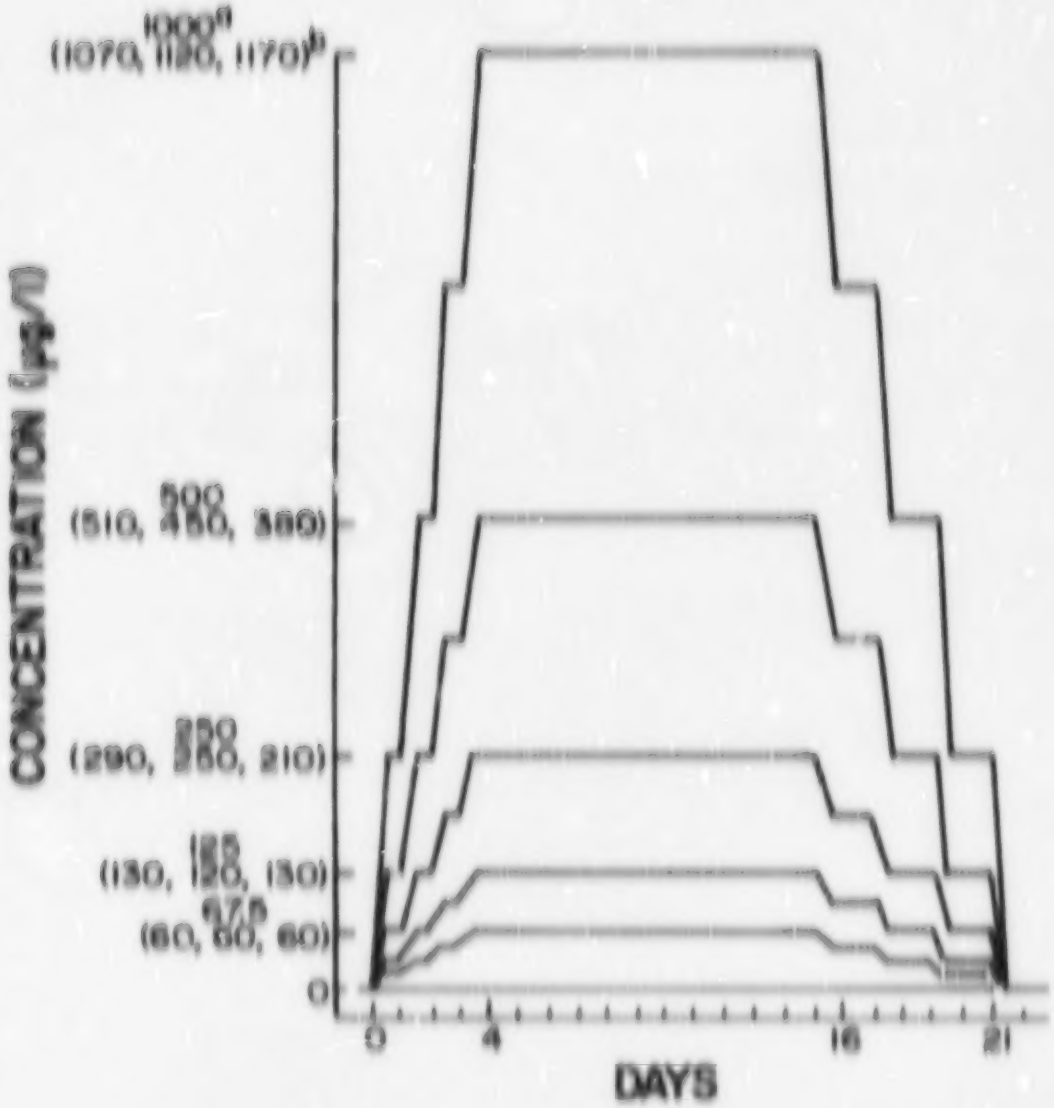


Figure 1. Schematic profiles of the diazinon concentrations administered in the larval-juvenile, juvenile-adult, and adult-spawning pulse exposures. [a]Nominal concentration. [b]Average measured concentrations in parentheses.

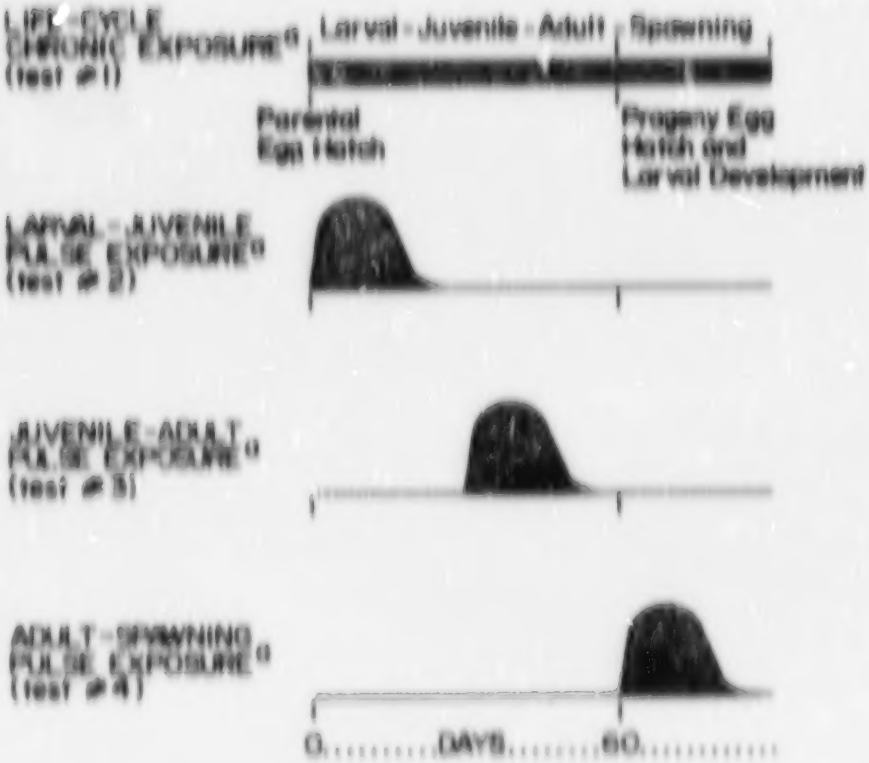


Figure 2. Schematic profiles of baseline-chronic and pulse exposure regimens. [a]Only one set of equivalent exposure units is represented for each test.

was eliminated by expressing results as a percentage of the respective control. Comparison was further simplified by combining all data for specific effects into a common index of overall impact on population success which has been named terminal biomass. Details of the calculation of terminal biomass are given in the appendix.

## SECTION 5

## RESULTS

### TERMINAL BIOMASS IN PULSE AND BASELINE-CHRONIC EXPOSURES

Detailed results used to calculate terminal biomass are presented in appendix, Tables 1, 3, 4, and 5.

Response regressions of terminal biomass for the baseline chronic and pulse tests are shown in Figure 3. As explained in the appendix, a time factor of 90 days was chosen to calculate exposure units in the baseline chronic test. For *Jordanella floridae* this time period included the life stages normally exposed in standard "life-cycle" tests with fishes. To show that a precise time value is not critical for demonstrating similar response between the pulse exposures and the life-cycle baseline exposure, the computational effects of a range of arbitrary baseline time frames between 60 and 120 days have also been delineated in Figure 3. The four types of exposure had similar impact on the test populations when treatment was quantified by exposure units.

### SURVIVAL OF PARENTAL FISH IN PULSE, CHRONIC, AND ACUTE TESTS

In this study death of parental stock was limited to the actual exposure period in the pulse tests and to the pre-reproductive period in the baseline chronic test. This loss of breeding stock was a major factor influencing reproductive potential and estimates of terminal biomass. It seemed likely that deaths from acutely toxic exposure before the reproductive period would have a similar influence on population success. Calculations were therefore carried out to determine if exposure-unit relationship would hold true for exposures of short duration.

Four-day acute data were available for flagfish from a previous study (Allison and Hermanutz, unpublished data). Baseline chronic exposure units for comparison of parental deaths were based on 60 days of pre-reproductive exposure as no deaths occurred beyond this age. The regressions of parental survival for pulse, chronic, and acute exposures (Figure 4) indicate that correlation of effects with exposure units may have some validity in exposures as short as 4 days.

Calculations of parental survival in acute and chronic exposures of fathead minnows and brook trout were based on unpublished data from Allison and Hermanutz (appendix, Table 6). Although chronic data for survival below 75% were not available, there is some indication that the exposure-unit relationship may hold true for these species also.

Figure 3. Estimated terminal biomass after one and a half generations expressed as percentage of control.

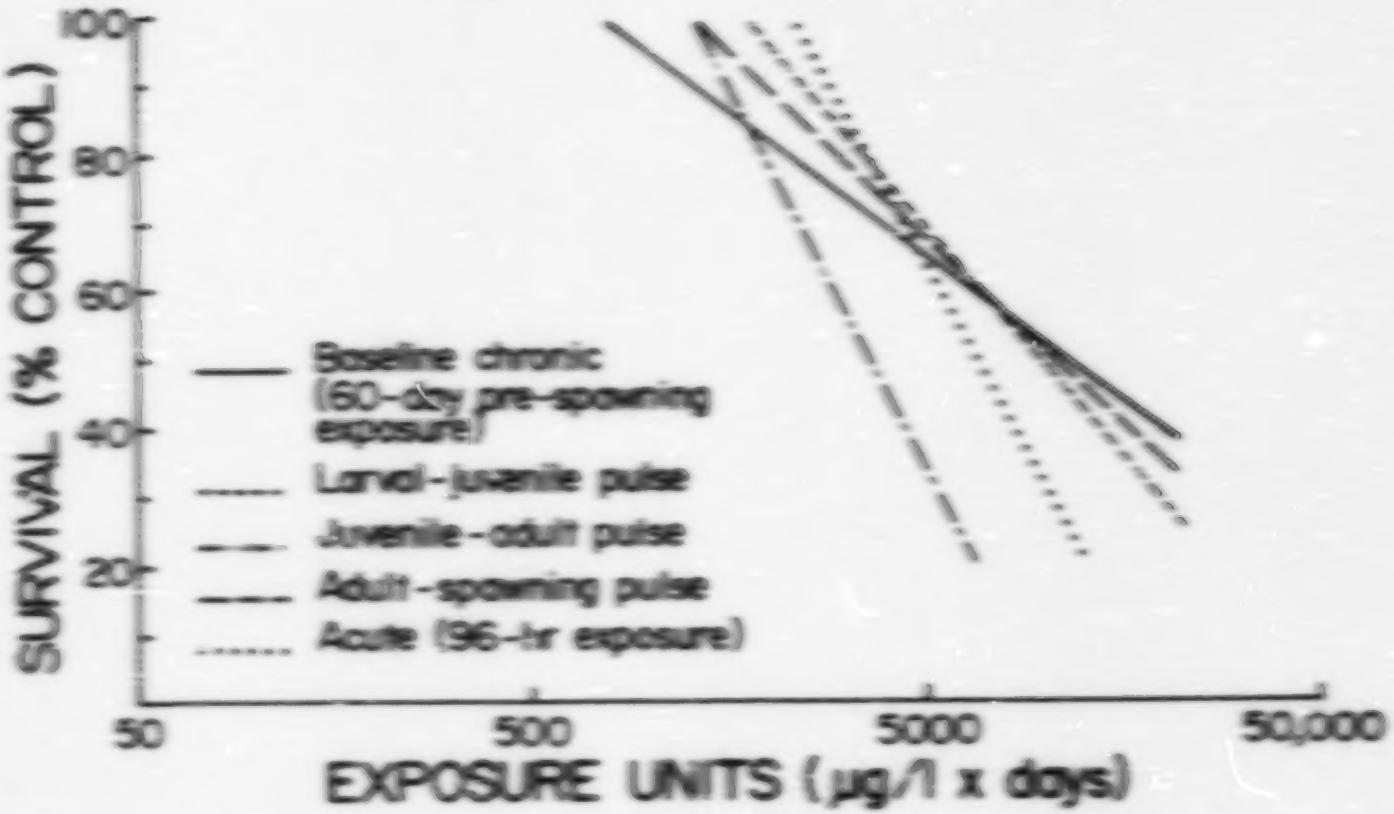


Figure 4. Survival of juvenile fish to reproductive age expressed as percentage of control.

## SECTION 6

## DISCUSSION

This study was designed as a preliminary investigation of the possible relationship of exposure concentration, duration, and periodicity to the toxicity of organophosphate pesticides. If very different degrees of chronic response follow exposures of distinct life stages or intermittent versus continuous exposure, these factors should be considered when evaluating the environmental impact of non-persistent pesticides. The single-pulse exposure used here was intermediate to the acute and chronic exposures currently used to derive water-quality criteria. The pulse tests also investigated the impact on population success of exposure limited to three different periods in the life cycle of a freshwater fish.

Many of the specific effects accepted as significant in laboratory bioassays cannot be correlated with overall survival of a population. It was not the intent of this study to demonstrate differences in degree of sensitivity for specific observed effects. The objective was to determine if different patterns of exposure resulted in variations in impact on population success. This study did not indicate any extreme population sensitivity to any profile or timing of exposure. Instead response was correlated rather closely to the product of exposure concentration and exposure duration ("exposure units") over a wide range of time factors. However, there are obvious time and concentration constraints on the use of exposure units. Validity must at some point diminish on approaching very high or low concentrations for very short or long durations respectively.

Subjective evaluation would be required to determine the advisability of initiating exposure during a breeding season. Spawning was temporarily inhibited in the adult-spawning exposure (see appendix), but terminal biomass of Jordanella was not greatly affected. However, if the physical and biological conditions necessary for successful reproduction in another species are of limited duration, an entire year class might be severely damaged by inhibition of spawning. Allison and Hermanutz (unpublished data) observed that brook trout, severely distressed for several weeks after initial exposure to diazinon, recovered and were capable of spawning after 6 months of continuous exposure. Although the continuous exposure did appear to cause some attrition, subjection of the trout to the same concentrations without acclimation just before their limited spawning period might have been more damaging to productivity.

In summary, the exposure-unit technique may have value in estimating the environmental impact of non-persistent pesticides over a range of exposure conditions. Estimated exposure units of a potential field situation could be summed and compared to exposure units of field or laboratory studies for which

results were already available. The concept should be verified with other species and toxicants and its limitations established and recognized before it is put to general use. In spite of restrictions it seems possible that the hypothesis may well be validated with sufficient confidence to warrant its inclusion in management decisions based on mass-balance modeling of water pollution.

## SECTION 7

## REFERENCES


Allison, D., B. J. Hallman, and G. B. Cope. 1963. Insecticides, effects on cutthroat trout of repeated exposure to DDT. Science 142:958-961.

Bradshaw, J. S., S. L. Loveridge, E. F. Rippee, J. L. Peterson, D. A. White, and D. E. Fuhriman. 1972. Seasonal variations in residues of chlorinated hydrocarbon pesticides in waters of the Utah Lake drainage system, 1970-1971. Pest. Monit. J. 6:166-170.

Brown, V. M., D. H. M. Jordan, and B. A. Tiller. 1969. The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. J. Fish Biol. 1:1-9.

Cowart, R. P., F. L. Bonner, and E. A. Epps. 1971. Rate of hydrolysis of seven organophosphate pesticides. Bull. Environ. Contam. Toxicol. 6:231-234.

Eaton, J. G. 1970. Chronic malathion toxicity to bluegill (Lepomis macrochirus Rafinesque). Water Res. 4:673-684.

Gomaa, H. M., I. H. Suffet, and S. D. Faust. 1969. Kinetics of hydrolysis of diazinon and diazoxon. Residue Rev. 29:171-190.

Mount, D. I., and C. E. Stephan. 1967. A method of establishing acceptable toxicant limits for fish -- malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96:185-193.

Sethunathan, N., and M. D. Pathak. 1972. Increased biological hydrolysis of diazinon after repeated application in rice paddies. J. Agr. Food Chem. 20:586-589.

## APPENDIX

## DETAILS OF TEST TECHNIQUES AND RESULTS

### METHODS

#### Baseline Chronic Exposure

Flagfish (*Jordanella floridae*) were used as the test species. Their relatively short life cycle (about 2 months egg-to-egg) allowed this series of tests to be conducted sequentially within a period of 2 years.

A modification of the proportional diluter equalized solvent concentrations in all test tanks. Temperatures were maintained between 25.5 and 26.5 C. Diazinon concentrations were nominally doubled between levels. Five concentrations (14-140 ug/l) and the control were duplicated. Exposure began with thirty 1-day-old flagfish per duplicate. Survival and growth were determined at 35 days and at termination. Each duplicate was thinned to two mature males and five mature females just prior to spawning. Two yarn-covered spawning substrates were placed in each duplicate chamber at 60 days, and spawning commenced immediately. After 60 days of spawning parental fish were killed and weighed.

Egg production was recorded daily. If numbers permitted, one sample of 30 eggs per duplicate was retained each day to measure hatch success. Twenty newly hatched larvae were placed in each of two larval chambers per duplicate on a space-available basis and were reared for 35 days to measure growth and survival (eight larval groups per treatment level).

#### Pulse Exposures

The pulse exposures were run sequentially in the test system used for the baseline chronic. The same basic test techniques were used. Fish initially exposed at 29 and 66 days (juvenile-adult and adult-spawning) were raised as pooled stock before testing. Observation of progeny growth and survival was limited to four groups per treatment level in the larval-juvenile and juvenile-adult tests as exposure had been discontinued before the reproductive period. Progeny produced early in the adult-spawning exposure (four groups per level) were maintained for 21 days before being terminated to make space available for post-exposure progeny studies of 35 days of growth and survival.

Chemical Analysis

Water levels of diazinon were monitored weekly in the baseline chronic and daily during the 21-day pulse exposures. Concentrations were determined by gas chromatography and electron capture detector.

Statistical Analysis

This study was conceived as a preliminary investigation to determine if exposures of different duration and seasonal occurrence might cause major differences in population success. Evidence of wide discrepancy might then warrant more sophisticated and costly experimental designs to quantify the factors involved. The possibility of extraneous variables (due to genetic and physical differences) as well as an inherent problem in the test design of precisely defining the time frame for each effect precluded direct comparison by standardized methods of statistical analysis and the use of mathematically derived tests of significance.

Exposure in the baseline chronic test lasted about 120 days. However, all deaths of parental fish took place before reproduction at 60 days. In addition, prolonged exposure of adults during reproduction apparently did not cause any correlated increase in effects on progeny. Therefore, for the purpose of comparison, 60 days was chosen as the exposure duration for parental deaths, and 90 days (60 days of parental exposure plus 30 days of progeny exposure) was chosen to represent a baseline chronic exposure for this species. The baseline chronic exposure period also encompassed the relative duration of a standard life-cycle exposure for fishes. The five levels of challenge could then be approximately quantified as "exposure units" to be duplicated in the pulse exposures. This same problem of defining the true exposure duration causing specific effects existed in the pulse exposures. Consequently less confidence can be placed on the relationship of exposure units to specific effects than to overall impact on population from the entire exposure. Direct comparison and overall effects between these pulse tests is probably justified to the same extent as that usually made between different acute or chronic exposures with similar experimental designs. However, extrapolation of comparisons between baseline chronic, pulse, and acute exposures requires a greater degree of subjective evaluation.

The specific responses recorded in this study were parental survival and weight of survivors, egg production per spawning female and hatch success, and progeny survival and weight of survivors. These indices were used to derive an index of overall impact on population success, named terminal biomass. As a matter of convenience, terminal biomass for each treatment was computed and compared per each theoretical mating pair (one male and one female) present at the start of exposure. The average weights of parental males and females at test termination were multiplied by percentage parental survival, and the results were added to give an estimated index for parental biomass excluding the influence of thinning before reproduction. Progeny biomass was estimated by multiplying percentage parental survival to spawning by eggs per female by percentage hatch by percentage progeny survival to

termination by average weight of surviving progeny. The values for parental and progeny biomass were added for each treatment and expressed as a percentage of their respective test control. Extraneous variance due to sequential rather than simultaneous testing of the baseline and pulse exposures was thereby removed by equating all control results to 100%, and comparison could be made for population response versus exposure units.

Since actual exposure units delivered (determined by water analyses) differed somewhat between tests, comparison was made with calculated regressions of response. The regressions for the pulse tests are based on four data points, as the highest levels of exposure killed virtually all of the parental stock. Response within the range of the semilog plots presented in Figures 3 and 4 was fundamentally linear as represented.

Because true time factors in these tests could not be exactly defined, exposure-unit values must be considered as best-estimate approximations. Any tendency to assign unwarranted accuracy to the results should be avoided.

RESULTS

Specific results of the baseline chronic and 21-day pulse exposures are summarized in Tables 1 through 5 of this section. The response of progeny in the baseline test (Table 2) is of special interest. In previous tests with fathead minnows and brook trout (Allison and Hermanutz, unpublished data) all responses of the progeny at lower test concentrations could be attributed to the pre-spawning exposure of the parents. However, effects on flagfish progeny appear correlated to both parental and progeny exposure. In flagfish, progeny exposure alone apparently caused greater effects than parental exposure alone, but this evaluation is subjective because paired data could not be used in this case and analysis of unpaired data did not demonstrate a statistically significant difference. Diazinon also reduced the incubation time of flagfish eggs, an effect not seen with fathead minnows or brook trout.

Four samples of eggs per treatment were collected at the beginning of the adult-spawning test just before spawning was interrupted by the exposures. These eggs did not show any distinct evidence of reduced or early hatch as noted in the baseline chronic, even though the average concentrations experienced before hatch were generally higher than those to which eggs were continuously exposed in the baseline test. Graduated increase in concentration may have reduced toxicity, but more probably the sample size was simply too small to identify differences in response. During the remaining exposure period of 13 - 16 days following hatch of these eggs, the larvae displayed a range of responses equivalent to that of fish of the same age exposed during the larval-juvenile pulse test.

In the highest concentration of each pulse test the fish were subjected to about two-thirds their 96-hr LC50 during the 12-day pulse peak. Sixty fish per test were exposed at this level, and only one of the 180 survived. Survival in lower exposures was adequate for comparison of growth, survival, and reproductive success. The three pulse tests did differ in degree of response for specific indices. For example, parental stock survived best in the larval-juvenile exposure and poorest in the juvenile-adult exposure.

Subsequent egg production per female was higher in the juvenile-adult exposure, but survival of progeny was poorer. These apparent differences may simply reflect variance due to different stocks of fish or slightly different physical conditions in sequential tests. In any event, the differences tended to cancel out during calculation of terminal biomass.

One effect was noted that was unique for the life stage exposed. Exposure in the adult-spawning pulse test was delayed until all groups commenced spawning. Spawning ceased abruptly when the various concentration profiles reached about 130 µg/l. Fish exposed to no more than 130 µg/l for 12 days resumed spawning as soon as the concentration was reduced, but fish exposed to higher concentrations did not resume spawning until 1 - 2 weeks after concentrations were reduced below 130 µg/l. During the post-exposure period egg production in the treated groups equaled or exceeded the control. The temporary inhibition of spawning appears to be the result of initial exposure during the reproductive period. Fish in the baseline chronic exposed to about 240 µg/l since hatch showed no evidence of spawning inhibition.

The actual impact of spawning inhibition on a fish population would depend on the length of the spawning season of a given species. In the flagfish the 21-day pulse exposure resulted in only a temporary interruption. Terminal biomass was calculated from equally weighted intra-exposure plus post-exposure reproductive data versus post-exposure data alone. Both methods of calculation resulted in biomass regressions within the range of those derived for exposure during the other two life stages.

TABLE 1. CHRONIC EFFECTS ON FLAGFISH (*JORDANELLA FLORIDAE*) OF CONTINUOUS LIFE-CYCLE EXPOSURE TO DIAZINON

| | | | | | | |
|---|---|---|---|---|---|---|
| Average measured concentration (µg/l) | 340 | 88 | 54 | 26 | 14 | 0 |
| Exposure mid-range (µg/l x days) for exposure period of 60 – 120 days | 16,400 | 5,280 | 3,240 | 1,560 | 840 | 0 |
| | 28,800 | 10,560 | 6,480 | 3,120 | 1,680 | 0 |
| **Parental effects** | | | | | | |
| Survival to 35 days post hatch | 50% | 77% | 78% | 67% | 80% | 85% |
| Average length at 35 days post hatch (mm) | 17.7 | 17.7 | 19.3 | 19.6 | 19.5 | 21.0 |
| Survival to spawning (55 days post hatch) | 5% | 55% | 62% | 73% | 70% | 78% |
| Average weight of males at termination (g) | 1.6* | 3.3* | 3.4 | 3.7 | 4.2 | 4.3 |
| Average weight of females at termination (g) | 2.2 | 2.3 | 2.3 | 2.4 | 2.5 | 2.8 |
| **Embryo progeny effects** | | | | | | |
| Egg production per female parent | 72% | 1,174 | 966 | 1,334 | 1,036 | 1,340 |
| Hatch success of eggs | 40% | 55% | 60% | 6% | 60% | 66% |
| Average start of hatch (days) | 3.0* | 2.9* | 3.3* | 3.4 | 3.3 | 3.3 |
| Average end of hatch (days) | 4.3* | 4.3* | 4.3* | 4.5* | 4.6* | 4.9 |
| **Larval progeny effects** | | | | | | |
| Survival to 35 days post hatch | 72% | 73% | 66% | 85% | 96% | 85% |
| Average weight at 35 days post hatch (g) | 0.18* | 0.20 | 0.21 | 0.18* | 0.18* | 0.23 |

*Significantly different from control (P=0.05) using one-way analysis of variance and Jennett's procedure.

TABLE 2. RELATIVE EFFECTS ON FLAGFISH PROGENY OF CONTINUOUS DIAZINON EXPOSURE OF PARENTS AND PROGENY FROM PAIRED OBSERVATIONS OF SUBDIVIDED EGG SAMPLES

| Concentration (ug/l) | Exposure of both parents and progeny *versus* Exposure of parents only | Exposure of progeny only *versus* No exposure of parents or progeny |
|---|---|---|
| | **Start of hatch (days)** | |
| 240 | 2.1 vs 2.7[b] | 2.5 vs 3.5[b] |
| 88 | 2.2 vs 2.2 | 2.5 vs 3.8[b] |
| | **End of hatch (days)** | |
| 240 | 3.6 vs 4.8[b] | 4.0 vs 5.2[b] |
| 88 | [illegible] vs 4.9[b] | 4.4 vs 4.9[b] |
| | **Hatch success** | |
| 240 | 29% vs 46% | 38% vs 62% |
| 88 | 41% vs 52% | 47% vs 59% |
| | **Larval survival[a] - hatch to 35 days** | |
| 240 | 48% vs 92% | 40% vs 75% |
| 88 | 88% vs 92% | 92% vs 78% |
| | **Larval weight (g)[a] - 35 days post hatch** | |
| 240 | 0.40 vs 0.36 | 0.19 vs 0.30 |
| 88 | 0.26 vs 0.27 | 0.24 vs 0.28 |

[a]Average of two subsamples per treatment.

[b]Pairs statistically different (P=0.05) on the basis of "t" test. At least eight pairs were used for each comparison

TABLE 3. CHRONIC EFFECTS ON FLAGFISH (*JORDANELLA FLORIDAE*) OF EXPOSURE DURING LARVAL-JUVENILE STAGES OF PARENTS TO 21-DAY PULSE OF DIAZINON[a]

| | | | | | | |
|---|---|---|---|---|---|---|
| Exposure entire (µg/l x days) | 10,000 | 6,400 | 4,000 | 2,500 | 1,000 | 0 |
| Average 21-day peak concentration (µg/l) | 1,070 | 510 | 290 | 130 | 66 | 2 |
| **Parental effects** | | | | | | |
| Survival to 35 days post hatch | 25* | 57% | 87% | 93% | 78% | 87% |
| Average length at 35 days post hatch (mm) | — | 22.9* | 24.1 | 24.4 | 25.4 | 23.3 |
| Survival to spawning (30 days post hatch)[b] | 25* | 57% | 87% | 90% | 78% | 87% |
| Average weight of males at termination (g) | — | 4.3 | 5.7 | 5.5 | 4.9 | 5.0 |
| Average weight of females at termination (g) | — | 3.1 | 1.6* | 2.1 | 2.9* | 2.2 |
| **Embryo progeny effects** | | | | | | |
| Egg production per female parent | — | 667* | 925* | 1,314 | 1,310 | 1,577 |
| Hatch success of eggs | — | 47% | 45% | 56% | 60% | 58% |
| **Larval progeny effects** | | | | | | |
| Survival to 35 days post hatch | — | 85% | 49% | 94% | 85% | 92% |
| Average weight at 35 days post hatch (g) | — | 0.32 | 0.12 | 0.25 | 0.32 | 0.09 |

[a]Parental stock exposed between 1 and 21 days after hatch.

[b]Spawning began 30 days after termination of diazinon exposure.

*Significantly different from control (P=0.05) using one-way analysis of variance and Bassett's procedure.

TABLE 4. CHRONIC EFFECTS OF FLAGFISH (*JORDANELLA FLORIDAE*) OF EXPOSURE DURING JUVENILE-ADULT STAGE OF PARENTS TO 21-DAY PULSE OF DIAZINON[a]

| | | | | | | |
|---|---|---|---|---|---|---|
| Exposure units (µg/l x days) | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average 21-day peak concentration (µg/l) | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| *Parental effects* | | | | | | |
| Survival to spawning (37 days post hatch)[b] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average length 55 days post hatch (mm) | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average weight of males at termination (g) | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average weight of females at termination (g) | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| *Metro progeny effects* | | | | | | |
| Egg production per female parent | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Hatch success of eggs | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| *Larval progeny effects* | | | | | | |
| Survival to 35 days post hatch | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average weight at 35 days post hatch (g) | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |

[a]Parental stock exposed between 29 and 50 days after hatch.

[b]Spawning began 7 days after termination of diazinon exposure.

[c]Significantly different from control (P=0.05) using one-way analysis of variance and Bonnett's procedure.

TABLE 5. CHRONIC EFFECTS ON FLAGFISH (*JORDANELLA FLORIDAE*) OF EXPOSURE DURING ADULT-SPAWNING STAGE OF PARENTS TO 21-DAY PULSE OF DIAZINON[a]

| | | | | | | |
|---|---|---|---|---|---|---|
| Exposure [illegible] (µg/l x days) | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average 21-day pool concentration (µg/l) | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| **Parental effects** | | | | | | |
| Survival[b] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average weight of males at termination (g) | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average weight of females at termination (g) | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| **Mature progeny effects during exposure** | | | | | | |
| Egg production per pre-exposure female parent | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Batch success of eggs | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| **Larval progeny effects during exposure** | | | | | | |
| Survival to 21 days post-batch | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average weight at 21 days post-batch | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| **Post-exposure mature progeny effects** | | | | | | |
| Egg production per female parent[c] | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Batch success of eggs | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| **Post-exposure larval progeny effects** | | | | | | |
| Survival to 21 days post-batch | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| Average weight at 21 days post-batch (g) | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |

[a] Spawning began [illegible] days after batch. Exposure initiated at [illegible] days when all groups were spawning.

[b] Deaths of parental stock were limited to the 21-day exposure period.

[c] Over [illegible] days of spawning immediately following exposure plus 7 days of spawning 1 month after exposure was terminated.

[d] Significantly different from control (P<0.05) using one-way analysis of variance and Bonferroni's procedure.

TABLE 6. SURVIVAL OF BROOK TROUT AND FATHEAD MINNOWS VERSUS

EXPOSURE DOSES OF DIAZINON[a]

Brook trout

| 4-day exposure | | 90-day exposure | | 170-day exposure | |
|---|---|---|---|---|---|
| Exposure units[b] | Survival | Exposure units | Survival | Exposure units | Survival |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| [illegible] | [illegible] | | | [illegible] | [illegible] |
| [illegible] | [illegible] | | | | |
| [illegible] | [illegible] | | | | |
| [illegible] | [illegible] | | | | |

Fathead minnow

| 4-day exposure | | 70-day exposure[c] | |
|---|---|---|---|
| Exposure units | Survival | Exposure units | Survival |
| [illegible] | [illegible] | [illegible] | [illegible] |
| [illegible] | [illegible] | [illegible] | [illegible] |
| [illegible] | [illegible] | [illegible] | [illegible] |
| [illegible] | [illegible] | | |
| [illegible] | [illegible] | | |

[a]Unpublished data from Allison and Benemann.

[b]Up to 4 days.

[c]Survival ratios between 50th and 160th day of exposure. High mortality from causes unrelated to exposure concentration precluded use of data from first 50 days.